Quarterly Progress Report Number 7

Transcutaneous Analyte Measuring Methods
(TAMM Phase II)

Dr. Kenneth J. Schlager

Biotronics Technologies, Inc.

April-September, 1993

Naval Medical Research and Development Command

Quarterly Progress Report Number 7

Transcutaneous Analyte Measuring Methods
(TAMM Phase II)

Prepared by
Principal Investigator
Dr. Kenneth J. Schlager

October 4, 1993

Biotronics Technologies, Inc.
W226 N555B Eastmound Drive
Waukesha, WI 53186

April-September, 1993

Contract Number N00014-91-C-0190

Prepared for
Naval Medical Research and Development Command
Bethesda, Maryland

Table of Contents

List of Illustrations

Introduction

This document contains a comprehensive status report on the TAMM Phase II SBIR project at Biotronics Technologies. It is important to understand at this stage of TAMM development the strengths and weakness of current instrumentation and the design approach to overcoming current shortcomings. Optimism at Biotronics remains high. Even though the low signal-to-noise ratio (SNR) of TAMM I instrumentation (the BI-800 Array Analyzer) made signal processing difficult and limited measurement accuracy, encouraging test results were still achieved, especially on the last 73 patients tested in Milwaukee.

The BI-800 has been returned to Biotronics for repair by the Naval Health Research Center (NHRC) in San Diego. The fiber cable was broken by Navy personnel in testing experiments at NHRC. This fiber cable will be repaired, and the system will be returned to NHRC in about four weeks for preclinical testing.

The current test program at NHRC presents significant financial problems for Biotronics. As this program is not covered by the current Phase II SBIR contract, a CRADA agreement between Biotronics and NHRC would seem appropriate. Biotronics understands the Navy's position that "encouraging" preclinical test results at NHRC are necessary before Phase III Navy funding is appropriate. At the same time, as a small business, Biotronics must find a way to cover its expenses in these efforts. For this reason, the company is seeking outside financial support for its noninvasive blood chemistry program. Additional external funding will provide Biotronics with considerably more flexibility in bringing TAMM technology to practical application both in the military and the commercial marketplace.

Meanwhile, Biotronics is continuing the development of the field instrument due for delivery under the Phase II SBIR program. This development has been delayed for two primary reasons:

1. LED Array

   A lightweight, battery-operated, near infrared array spectrometer instrument is feasible only with light-emitting diodes (LEDs) as light sources. A halogen light source, as on the BI-800, would consume too much power for practical battery operation. To cover the specified near infrared spectral region, a special LED array had to be developed. The time required to develop this special LED array has delayed completion of the NIR field instrument until the November-December, 1993 time period. More information on the final delivery date will be provided as it becomes available.

2. Funding Limitations

   The limited funding remaining on the TAMM Phase II SBIR program has severely constrained Biotronics in the field instrument development.

The remaining sections of this report are dedicated to a comprehensive update on the technical status, problems and solutions in the TAMM program.

Project Status

Biotronics is currently in the final stages of a Phase II SBIR program sponsored by the Naval Medical Research and Development Command. TAMM technology has demonstrated great promise in early preclinical testing of over 500 patients in the Bethesda Naval Hospital and Milwaukee County Medical Complex. Despite this promise, another round of instrumentation development will be required to

provide a TAMM instrument with the signal-to-noise ratio (SNR) necessary for the required accuracy in clinical application. This status report reviews previous instrumentation development, preclinical data collection and signal processing/pattern recognition development with a view to highlighting both strengths and weaknesses of TAMM technology at the present time.

The next stage of development in both instrumentation and signal processing/pattern recognition is described in some detail. This technology will be embodied, insofar as possible, in the field instrument scheduled for delivery to the Navy in late 1993. The rationale for the design approach selected and how it addresses the problems experienced in early testing/evaluation are also presented.

Instrumentation

A block diagram of the NIR-800 Array Spectrometer is shown in Figure 1. A halogen light source is coupled to a fiber through a shutter and attenuator. The shutter allows for light blocking necessary for a dark reading of the photodetector array. Near infrared light in the 1100-1800 nm spectral region is transmitted to the reflective optical probe that is coupled to the skin surface. Diffuse back-scattered light, which penetrates the epidermis and dermis as shown in Figure 2, is collected by the receiving fiber that transmits the light to the spectrograph where it is detected by an indium gallium arsenide photodetector array (256 x 1) and converted to digital form in the array interface electronics. The spectral signals are then interfaced to a microcomputer for signal processing and pattern recognition.

A photograph of the NIR-800 is shown in Figure 3. The lower of the two black enclosures is the spectrograph, and the upper one is the light source module. The patient's arm is strapped down to the fiber probe on the arm of the chair. The PC-type microcomputer is along side of the NIR-800 modules.

Strengths and Weaknesses of TAMM I Instrumentation

The TAMM I instrument (NIR-800 Array Spectrometer) performed all the functions necessary to measure diffuse reflective near infrared light in a human patient and to process spectral data to estimate blood analyte concentrations. This instrument was based on a newly developed gallium arsenide photodiode detector array. This photodetector array and its associated electronics became the limiting element in the TAMM instrument. Its signal-to-noise ratio (SNR) limitations established the basic accuracy of TAMM diagnostic measurements. Future improvement of TAMM will depend on the removal of this photodetection SNR barrier. Although extensive efforts were made in both operating procedures and signal processing/pattern recognition to make the best use of measured data, the SNR of the photodetector array limited the degree of accuracy achievable with the system.

Aside from measurement accuracy, a second practical limitation in TAMM I was the employment of a fiber probe. The use of a fiber probe did provide a degree of flexibility in selecting the best body location for reflective near infrared measurements. This early advantage, however, was outweighed later by three major disadvantages:

1. Extensive light losses in the fiber further degraded the SNR;

2. Increased significantly the cost of the instrument; and

3. The fiber probe is subject to breakage and frequent need of replacement.

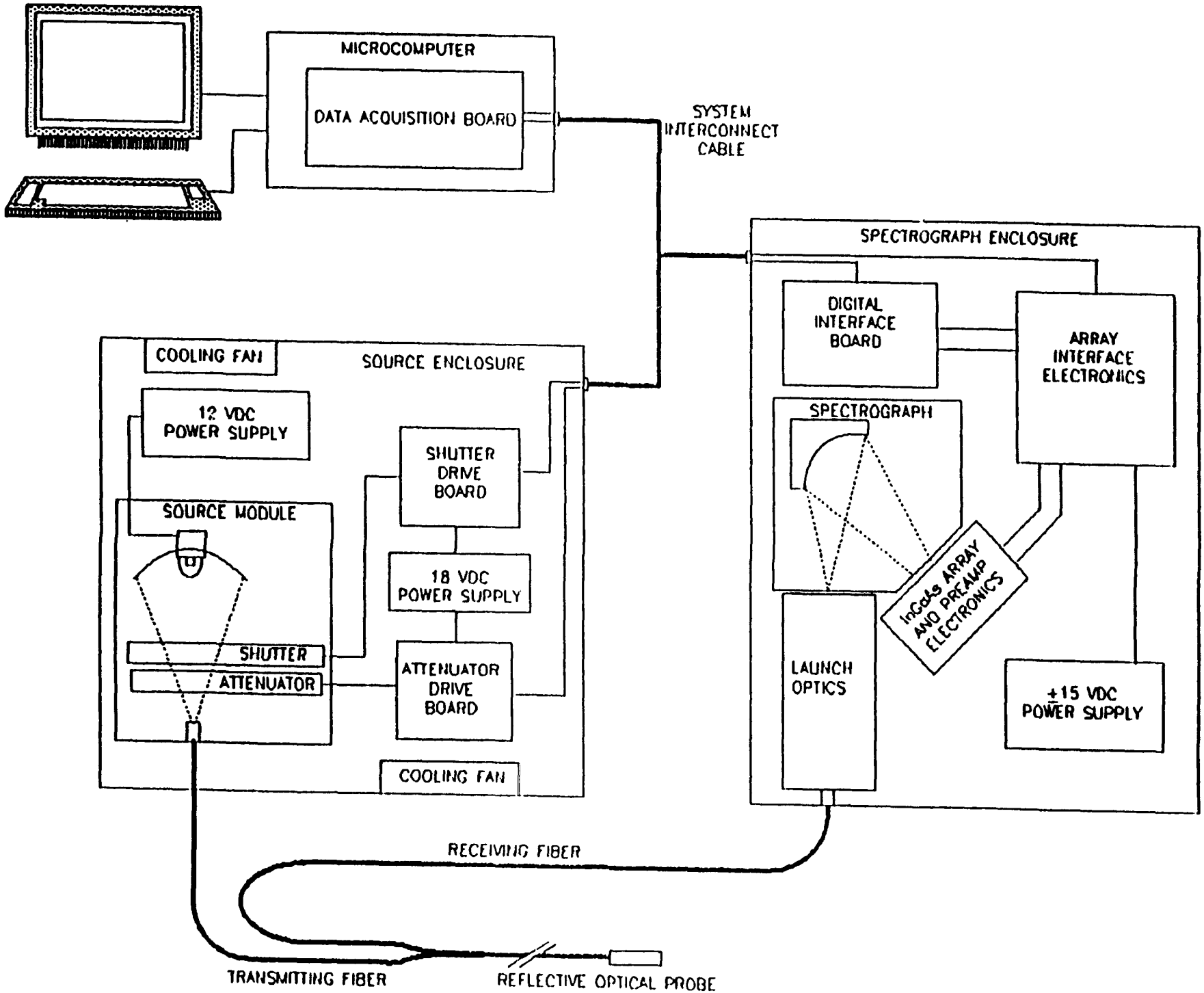

Figure 1. NIR-800 Array Spectrometer Block Diagram

OPTICAL PATHWAYS IN THE SKIN
(for near infrared reflectance analysis)

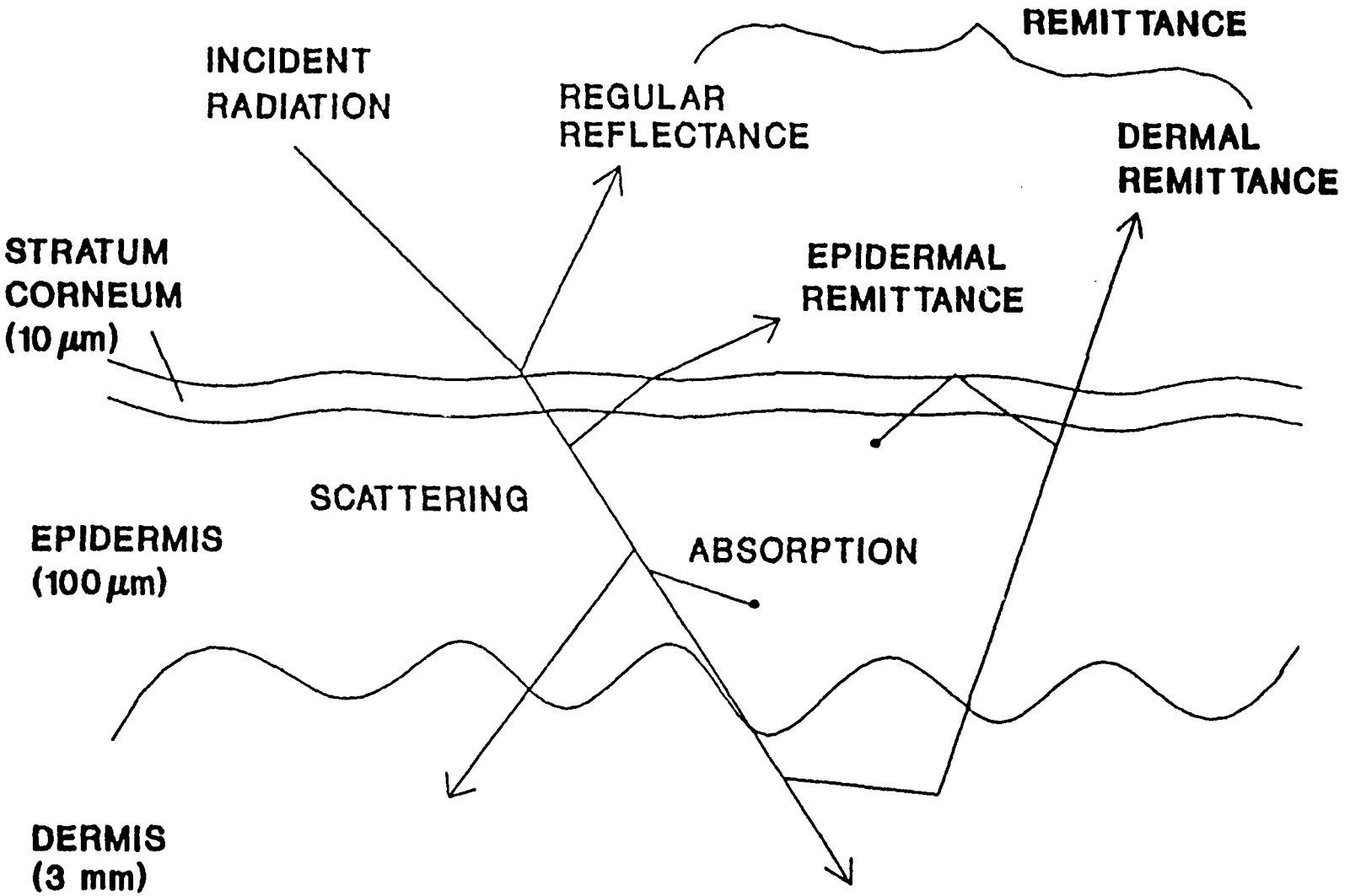

Figure 2.

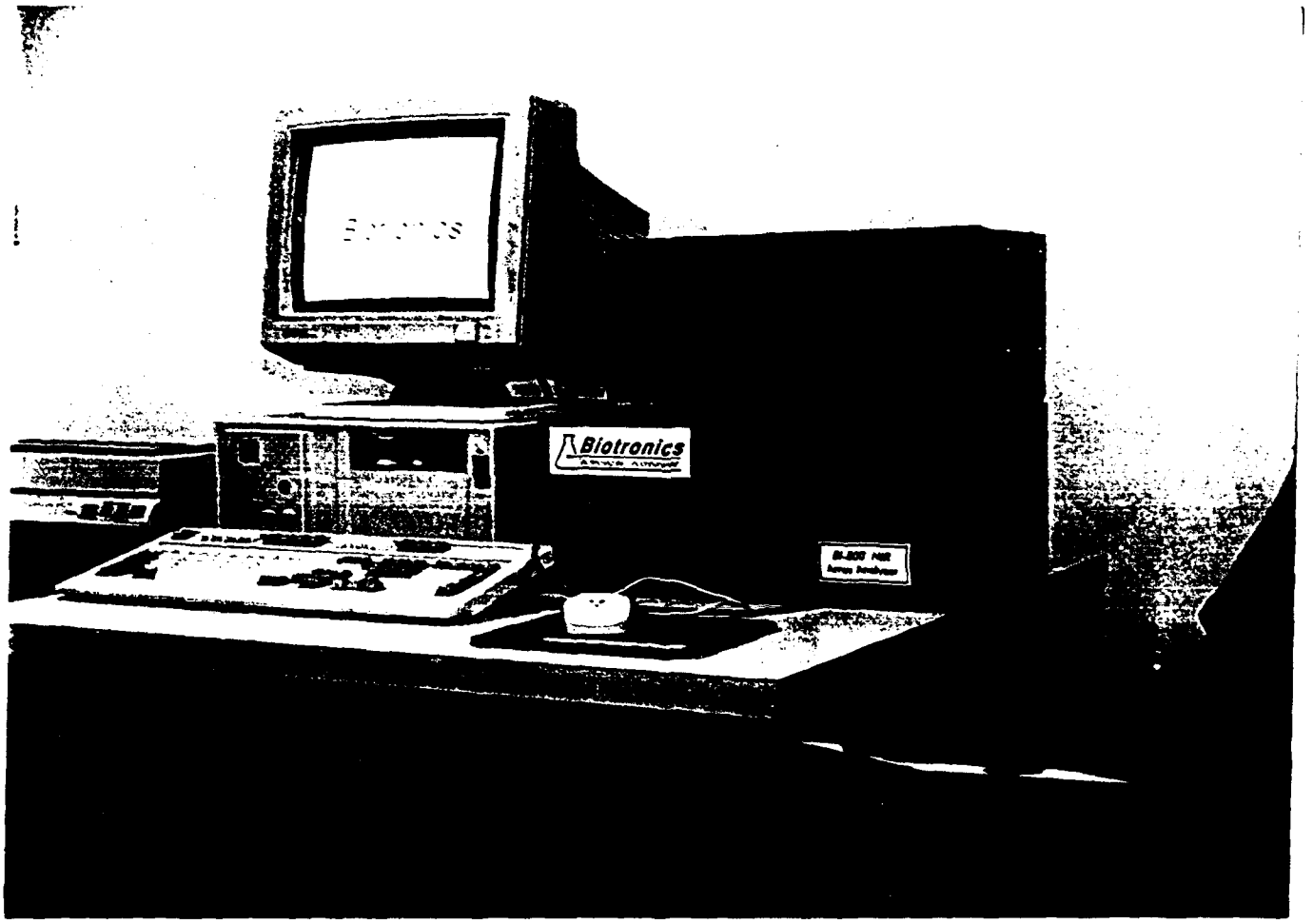

Figure 3. BI-800 NIR Array Analyzer

Finally, it should be emphasized that the TAMM I instrument was designed as a data collection instrument to gather patient data to evaluate the accuracy of near infrared spectrometry as a vehicle for noninvasive blood chemistry. Although the instrument was later converted at the Navy's request to a diagnostic instrument, it was never designed to perform diagnostic measurements in a medical environment.

Preclinical Database

Preclinical data collection was a learning experience. A primary goal of this data collection, aside from accuracy evaluation, was instrument/procedural optimization including the following:

1. Photodetector integration time;

2. Light/dark scan sequence; and

3. Probe positioning - mobile or fixed.

A breakdown of the TAMM database is shown in Table 1. The database is classified into 5 data subsets based on the test location, calendar dates and operational parameters. The first 249 patients were tested at the Bethesda Naval Hospital in Bethesda, Maryland. The second 265 patients were outpatients at Froedtert Memorial Lutheran Hospital in Milwaukee, Wisconsin. The start and stop dates for each data subset are also indicated in Table 1. Aside from location and dates, the subsets differed primarily in the operational parameters employed. System optimization was not achieved until data subset 5, based on the following selected parameters:

1. Probe Position

   A fixed probe with the arm strapped down was found necessary as opposed to a hand-held fiber probe.

2. Integration Time

   An integration time of 150 milliseconds was found optimal to achieve the best SNR.

3. Scan Sequence

   A 90 light, 90 dark scan sequence minimized instrument drift and noise.

Unfortunately, this instrument/procedural optimization provided only 73 data samples with the highest SNR. Although it is possible to evaluate the instrumentation and signal processing/pattern recognition algorithms based on 514 patients, better results are achieved with the final 73 samples.

Another weakness of this database is the limited range of the analyte concentrations. Bethesda patients were healthy military personnel undergoing annual physical examinations. For this reason, with rare exceptions, blood analyte concentrations were in the normal ranges. These ranges are indicated in Table 2 for each analyte. Froedtert hospital patients were persons with various medical problems in an outpatient environment, but again, the range of blood analyte concentration was limited. It now appears that data from an emergency or intensive care environment will be necessary to extend the range of blood analyte concentrations.

The use of this database in determining blood analyte values is discussed in the section that follows.

Data Subset	Hospital	Start Date	Stop Date	Probe Position	Integration Time (milli-sec.)	Sequence Scan	Accepted	Collected
1	Bethesda	6/23/92	7/1/92	Mobile	100	10/10	126	126
2	Bethesda	7/21/92	8/5/92	Mobile	60	10/10	123	124
3	Froedtert	9/09/92	10/23/92	Mobile	60	10/10	118	122
4	Froedtert	10/28/92	12/4/92	Mobile	60	90/90	74	80
5	Froedtert	1/20/93	2/15/93	Fixed	150/250	90/90	73	79
						TOTAL	514	531

Table 1. TAMM Database

Analyte	Mean Value	Range	Average Error	Slope (b)	T
Calcium	9.63 mg/dl	8.5-10.6	0.21 mg/dl 2.18%	0.57	7.01
Potassium	4.44 mmol/L	3.5-5.5	0.16 mmol/L 3.60%	0.75	10.82
Sodium	140.51 mmol/L	135-148	1.24 mmol/L 0.88%	0.59	6.95
Chloride	103.68 mmol/L	94-110	1.13 mmol/L 1.09%	0.57	9.56
Bicarbonate (CO_2)	28.56 mmol/L	22-35	1.55 mmol/L 5.43%	0.46	4.12
Glucose	98.07 mg/dl	60-115	13.97 mg/dl 14.24%	1.00	14.61
Urea (BUN)	15.58 mg/dl	7-26	1.99 mg/dl 12.77%	0.67	9.17
Hematocrit	41.78%	33-55	2.62% 6.27% of mean	0.41	5.94
Hemoglobin	14.32 g/dl	12-18	0.89 g/dl 6.22%	0.48	6.45

Table 2. Analytical Results (TAMM)

Notes:

1. An effective measurement is characterized by small average error, a slope approaching 1.0 and a high T-value. The slope is defined as the tangent of the least square fit line of predicted values/actual values.

2. The T-value can be described as a "tracking value" that indicates how well the predicted value tracked with changes in the actual value. It is defined by the slope/standard error of the slope.

Preclinical Test Results, Signal Processing and Pattern Recognition

A dual approach to explaining the results of test data analysis will be pursued in this section:

1. **Rationale for Previous Test Results**

   - **obtained using the NETGEN genetic neural network program**

2. **Demonstration of simple functional relationships existing between near infrared spectral absorbance and analyte concentrations.**

The first approach is difficult because a neural network software package is by its nature a "black box" in which spectral data inputs are received and analyte concentrations are produced. Like most pattern recognition algorithms, a neural network operates in two modes:

1. **Learning (training) phase; and**

2. **Test phase.**

During the learning phase, labeled samples along with their associated spectra are "fed" to the neural network which "learns" the appropriate parameters to accurately predict the labeled sample concentrations. During the test phase, these parameters are then used to predict the chemical concentrations of a second set of samples. These predictions are then compared with the laboratory-measured values to determine the accuracy of the instrument. The average error, slope and T-values of these predictions are shown in Table 2. The slope and T-value terms are defined in the table. Slope is a measure of how well the prediction "tracks" an analyte, and T-value is a test of whether these results could have occurred by chance. All T-values are significantly above the chance level ($p = 0.001$ or better).

Average error computations were based on comparisons with standard laboratory assays using blood samples extracted at the time of the near infrared spectral measurement. Laboratory assays were performed by the Navy clinical laboratories in Bethesda and by Roche Diagnostics at Froedtert in Milwaukee.

Because of the variations in integration time, it is not possible to analyze the total database as one entity. Instead, the database must be analyzed in database subsets of equal integration time. A longer integration time would produce a higher signal level for the same light absorbance. Variations in scan sequence will not change the basic signal, but more extensive instrument short-term drift was observed with the 10/10 sequence. The mobile probe option produced significantly greater noise in patient measurements. It proved difficult for the operator to hold the probe steady during the scan sequence.

Despite the inherent noise characteristics of the indium gallium arsenide photodetector array, the data set 5 combination of a fixed probe, 150 millisecond integration and 90/90 scan sequence produced a quite effective measurement environment. For this reason, pattern recognition analysis concentrated on this data subset. The superior quality of this data subset was consistently demonstrated in comparative NETGEN analyses, and microscopic analyses of individual analytes, shown later in this section, will clearly confirm this difference in quality.

NETGEN Neural Network Analysis

It would be inappropriate in this report to provide a tutorial on neural networks and their application to spectral pattern recognition. A detailed description is provided in the NETGEN technical manual that is included with this report. This manual describes both the theory and application of a genetic algorithm-assisted neural network to spectral pattern recognition. Here, the commentary will be limited to NETGEN test results.

The difficulty of interpreting the performance of neural network-based pattern recognition is that the network operates as a holistic black box. The role of the genetic algorithm in NETGEN further complicates results interpretation. Commentary here will be limited to the external inputs. The 73-patient data set was divided equally into training and test sets. NETGEN was trained with the training set and tested with the test set. There is a significant number of operator-controlled parameters in NETGEN application. A skillful operator can improve results considerably through experimentation. NETGEN analysis, then, typically consists of a series of runs in which the parameters are varied to optimize test results. The values shown in Table 2 are the results of such a series of NETGEN runs on the 73-patient database.

Microscopic Analysis of Selected Analytes

Realizing the difficulty of interpreting the validity of neural network predictions of blood analysis, detailed microscopic analyses of 6 of the 9 analytes have been carried-out. These analyses confirm the basic predictive value of reflective near infrared spectral information in a format that is direct and easy to understand. The objective of these analyses was to search out and verify a single wavelength in which spectral absorbance bore a simple relationship to analyte concentration. Such a demonstration would strengthen the credibility of TAMM particularly in the minds of observers unfamiliar with the technology of neural network analysis. This demonstration, however, should not imply that such a simple relationship represents the optimal method of analyte concentration estimation. The objective of the demonstration was solely to convince the skeptical of the presence of predictive information for noninvasive blood chemistry in near infrared spectra.

The procedures followed in this microscopic spectral analysis are detailed below:

1. Spectral Range

   The spectral range was limited to the 1250-1350 nm region. If useful and complete information can be found in this region, it will greatly simplify the final instrument design and significantly improve its performance.

2. Spectral Absorbance Sort

   All 73 samples (except for glucose and BUN with 62 and 64 samples, respectively), were sorted in ascending spectral absorbance order at a selected wavelength.

3. Absorbance Bin Classification

   Given the noise level of the system (absorbance noise = 0.01-0.02 absorbance units), indicated absorbance values were classified into bins or buckets of approximately equal

   absorbance within the noise band. These bins ranged from 0.01 to 0.02 absorbance units in size.

4. **Average Bin Analyte Concentration Level**

   The average value of analyte concentration was then determined for each bin.

5. **Linear Regression Function**

   The average absorbance in each of the bins was then regressed against the average analyte concentration for all of the bins.

6. **Evaluation**

   Results of the regression were evaluated based on the coefficient of determination (R^2) for each analyte at each wavelength.

It is quite apparent that a great deal of smoothing is going on here. Given the low SNR (5-10 dB), there is no choice but to average in order to reduce the uncertainty of the acknowledged noisy data. The results of these microanalyses are summarized in Table 3.

The results of this series of microanalyses are really quite remarkable considering the known spectral interferences between analytes. All of the R^2 are above 0.7 indicating that 70% or more of the absorbance variation is "explained" by the variation of the analyte concentration. The wavelengths at which the above predictions were made vary with each analyte but are not indicated here for proprietary reasons. Although it is highly unlikely that a single wavelength will be used in the final algorithm, it is truly amazing that a single wavelength should contain so much information about one analyte. The T-values are all sufficiently high that the null hypothesis of no relationship is very unlikely at the p = 0.001 or better. The standard (root mean square) estimation errors are also very respectable, better in most instances than the neural network predictions, and the slopes, except for the case of glucose, are also higher.

The remaining three analytes bicarbonate, hematocrit and hemoglobin did not have a good predictive single wavelength in the 1250-1350 nm spectral region.

Microanalysis of the Other Data Subsets

The superior quality of the fifth data subset has been previously emphasized based on NETGEN analyses. To further verify this qualitative difference, microanalyses were performed for the same 6 analytes using data from the other 4 data subsets. The results were very revealing. With the exception of calcium and sodium, not a single analyte demonstrated an R^2 above 0.5 in the other 4 data sets indicating that the SNR was significantly improved in the fifth data subset.

TAMM II Instrumentation Development

TAMM I instrumentation (the NIR-800 Array Analyzer) did not provide adequate signal-to-noise ratio (SNR) performance to take advantage of the reflective information present in chemical near infrared spectra. TAMM II instrumentation will be designed to significantly increase SNR performance while at the same time reducing the size, weight and power requirements for field military application. The design approach being used in TAMM II instrumentation development involves the following:

1. **Lock-In Signal Detection of Near Infrared Signals**

   Earlier near infrared spectrometers developed by Biotronics were based on chopper-modulated lock-in detection using a moving monochromator and a single photodetector. With the switch

Analyte	Std. Error Estimate	N	R^2	Slope	Std. Error Slope	T-value	P
Sodium	0.70 mmol/L 0.4%	73	0.74	0.74	0.16	4.62	0.001
Chloride	0.84 mmol/L 0.8%	73	0.70	0.70	0.17	4.11	0.001
Calcium	0.03 mg/dl 0.3%	73	0.85	0.85	0.16	5.31	0.001
Potas-sium	0.11 mmol/L 2.4%	73	0.78	0.78	0.17	4.59	0.001
Glucose	[illegible] mg/dl 2.9%	62	0.76	0.76	0.17	4.47	0.001
BUN (urea nitrogen)	0.58 mg/dl 3.7%	64	0.75	0.75	0.19	3.95	0.001
Choles-terol	15.52 mg/dl 7.1%	73	0.76	0.76	0.17	4.47	0.001
Trigly-cerides	19.34 mg/dl 9.7%	73	0.82	0.82	0.16	5.12	0.001

NOTE 1: The reduced samples used on glucose and BUN arose not so much from outliers as isolated low and high values too few in number to form a bin.

NOTE 2: Cholesterol and triglycerides were not part of the original 9 Navy analytes but were added later because of their obvious clinical importance.

Table 3. Single Wavelength Microanalyses

to self-scanned photodetector arrays, integration replaced lock-in detection as the method of signal processing. AlGaAs self-scanned arrays in the near infrared proved to be noisy, and integration (considering dark noise limitations) did not provide the same low-level detection capability as lock-in detection.

2. Modulated Light-Emitting Diode (LED) Array Light Source

   Use of a chopped halogen lamp for lock-in detection is not consistent with the need for a lightweight, battery-operated field instrument for combat casualty care for the military (Navy/Marine Corps). LED-array light sources may be electronically modulated without the need for a mechanical chopper. They are also lightweight, low in power consumption and generally available in the 1150-1350 nm spectral range.

3. Parallel Output Photodetector Arrays

   Operating in a more restricted spectral range allows for the use of smaller parallel output photodetector arrays. These arrays in sizes of 16 x 1 or 32 x 1 allow for lock-in detection in conjunction with a modulated LED light source array.

4. Direct Optical Readout (Non-Fiber Probe) Operation

   The use of a fiber probe proved extremely useful in the early experimental phase of TAMM I development. Various body locations could be explored as potential reflective measurement points. With the forearm body location fixed, however, the fiber probe becomes a liability with its ease of breakage, high light losses and high cost. A direct optical measurement of the forearm avoids all of these problems.

The specification for the LED/parallel output array TAMM II instrument is included in Appendix I.

Findings, Conclusions and Recommendations

TAMM I instrumentation in the form of the BI-800 NIR Array Spectrometer demonstrated the basic feasibility of measuring transcutaneous blood chemistry despite the low signal-to-noise ratio (SNR) resulting from the use of a new near infrared photodetector array. Optimization of operational parameters and a fixed, "strapped down" fiber probe were necessary to achieve acceptable accuracy. Analyte concentration estimates were based on the employment of a neural network aided by a genetic algorithm. It was also possible, however, to demonstrate simple and accurate linear relationships between absorbance and analyte concentrations at selected wavelengths.

Conclusions

With the basic feasibility of transcutaneous, reflective near infrared blood chemistry established, it is now necessary to "move on" to the new, pulsed LED/parallel output array with lock-in signal detection. This new design has the potential for a significant improvement in SNR over TAMM I instrumentation. But even with the current SNR, the employment of lock-in detection allows for accurate measurements at much lower SNRs. The new design will also be more reliable with an LED-array light source and direct (non-fiber) optical readout. Improved SNR will also allow for the use of a variety of pattern recognition techniques including adaptive methods that do not require large databases.

Recommendation

TAMM II instrumentation development should move forward based on the lessons learned in previous preclinical testing. Such a TAMM II instrument should provide the basis for FDA approval and a launch of the product into the clinical marketplace.

Kenneth J. Schlager
Principal Investigator

Appendix I

Subj: Specifications: TAMM Near Infrared Spectrometer for Military Application in the Field (The TAMM Field Instrument) (TAMM Field Prototype)

Date: February 8, 1993

1. General Specifications

1.1 Operating Principle

The TAMM Field Prototype is a near infrared, absorption spectrometric instrument intended to demonstrate the concept of a portable battery-operated instrument for noninvasive blood chemistry analysis in a military field environment. Reflective near infrared absorbance measurements in the human forearm area will provide variable values used in pattern recognition algorithms that determine estimated chemical concentrations of a variety of blood analytes. Light measurements are converted to digital form and processed through pattern recognition algorithms in a computer to provide measurements of 9 blood analytes.

1.2 Spectral Operating Range

1150-1310 nm

1.3 Modes of Operation

1. Diagnostic

    a. measures near infrared light absorption
    b. calculates blood analytes concentrations
    c. displays concentrations (printing optional)

2. Calibration

    a. measures reflectance standard
    b. measures wavelength standard
    c. adjusts instrument calibration

1.4 Analytical Algorithms

1. Genetic neural network analysis (NETGEN)

2. Adaptive signal processing and pattern recognition

1.5 Blood Analytes

1. Sodium
2. Calcium
3. Chloride
4. Potassium
5. Carbon Dioxide
6. Glucose
7. Urea
8. Hematocrit
9. Hemoglobin

2. Inputs

2.1 Optical

Near infrared reflectance measurements of human forearm.

Dynamic range - Absorbance - 0-1.4 AUs

2.2 Temperature

Contact skin temperature of human forearm in reflectance target area.

2.3 Operator Interface

a. mode selection
b. analyte selection
c. numeric patient identification
d. other operator inputs

3. Outputs

3.1 Digital Display

3.1.1 Operator prompts

3.1.2 Analyte concentrations

3.2 Printer (Optional Output)

3.2.1 Analyte concentrations and patient information

4. Instrumentation (Hardware)

4.1 Light Source

Light source in the wavelength range of 1150- 1310 nm with performance characteristics suitable for the requirements of 5.0. Irradiance level should not exceed 150 mw/cm^2.

4.2 Optical Configuration

Light will be transmitted to instrument arm rest. Reflected light will be collected and transmitted back for photodetection, digital conversion and processing. Optics and photodetection will be designed to provide a resolution of 5.0 nm and wavelength stability of $\pm$2.5 nm.

4.3 Analog-Digital Conversion

Successive approximation converter

14-bit resolution

4.4 Microcomputer

Processing power must be sufficient to perform measurement and analysis within a maximum time period of 20 seconds.

Program and algorithm parameters in EPROM or RAM memory

No hard drive

No diskette "floppy" drive

4.5 Display

40 x 20 liquid crystal display (LCD)

4.6 Power Supplies

As required.

4.7 Temperature Controller

Temperature control should be provided only as required for accurate measurement and only as possible with battery operation.

4.8 Enclosure

A small, compact enclosure that lends itself to being carried on a backpack frame by a Navy Corpsman is desired.

4.9 Power Source Requirements - Battery Operation

Rechargeable battery operation is an absolute requirement of this instrument. Recharge will be accomplished from a separate battery pack. Storage capacity will provide initially for the analysis of a minimum of 10 patients before recharge.

4.10 Weight

The instrument must weigh less than 20 pounds (excluding optional printer).

5. Performance

5.1 Light Measurement Accuracy

Absorbance - $\pm$0.1 MAU (milliabsorbance units) standard deviation of mean measurement.

5.2 Instrument Accuracy

This instrument will incorporate algorithms developed using the NIR-800 Array Analyzer. Average error, slope and T-value will be consistent with performance on the NIR-800. Algorithm development is not part of this specification.

5.3 Measurement Time

Measurement time will be limited to 10 seconds.